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Serial ultrathin sectioning demonstrating the intracellularity of *T. pallidum*

An electron microscopic study

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The possibility that *Treponema pallidum* could become engulfed within the cytoplasm of a host cell was suggested when early light microscope findings presented the appearance of intracellularity (Sauvage and Levaditi, 1906; Nyka, 1934; Nicol, Rios-Montenegro, and Smith, 1969). Several investigators, using ultrathin sectioning and electron microscopy techniques, which eliminate those artefacts of light microscopy which are due to tissue thickness and low resolution, have also reported finding treponemes which appeared to be enclosed within the cytoplasm of cells because of their proximity to mitochondria, Golgi apparatus, and intracellular vacuoles (Abe, 1967; Azar, Pham, and Kurban, 1970; Ovcinnikov, and Delektorskij, 1969b; Goldman, 1969, 1970, 1971).

But, as suggested by Goldman (1969, 1971), the possibility that a treponeme could be located within an invagination of the host cell membrane or bebetween folds of cytoplasm must be considered in evaluating the possibility that *Treponema pallidum* might occupy an intracellular habitat. Serial sectioning eliminates the one-dimensional view of the single section and allows a more accurate interpretation of the relationship of the treponeme to its apparent host cell.

This is a report of studies of experimental rabbit orchitis caused by *Treponema pallidum*. The organism has been shown to invade a variety of host cell types, some of which are generally considered to be phagocytic.

Material and methods

Approximately 10^8 Nichols 1 strain *Treponema pallidum* were inoculated into each testis of male New Zealand rabbits and allowed to develop a firm orchitis (6–13 days) The animals were killed with chloroform and 1 × 1mm.^{3} pieces of testis were fixed in one of two fixatives: chrome osmium (Dalton, 1955; Glauert, 1965) or 3 per cent. gluteraldehyde in cacodylate buffer and post-fixed in buffered 2 per cent. osmium tetroxide (Sabatini, Bensch, and Barrnett, 1963). After initial dehydration in a series of ethyl alcohols of increasing concentration, the tissues which had been fixed with Dalton's chrome-osmium fixative were stained *en bloc* with 20 per cent. uranyl acetate. All tissues were finally dehydrated in propylene oxide and embedded in the epon mixture of Luft (1961). The blocks of tissue were sectioned on an LKB Ultrotome III and picked up on 2 × 1 mm.^{2} single slot grids covered with formvar and carbon films. The sections were stained with lead citrate (Reynolds, 1963) for 10 minutes or double-stained (Frasca and Parks, 1965) with uranyl acetate (15 min.) and lead citrate (5 min.). AEI 6B and 801 electron microscopes were used for observation and photography.

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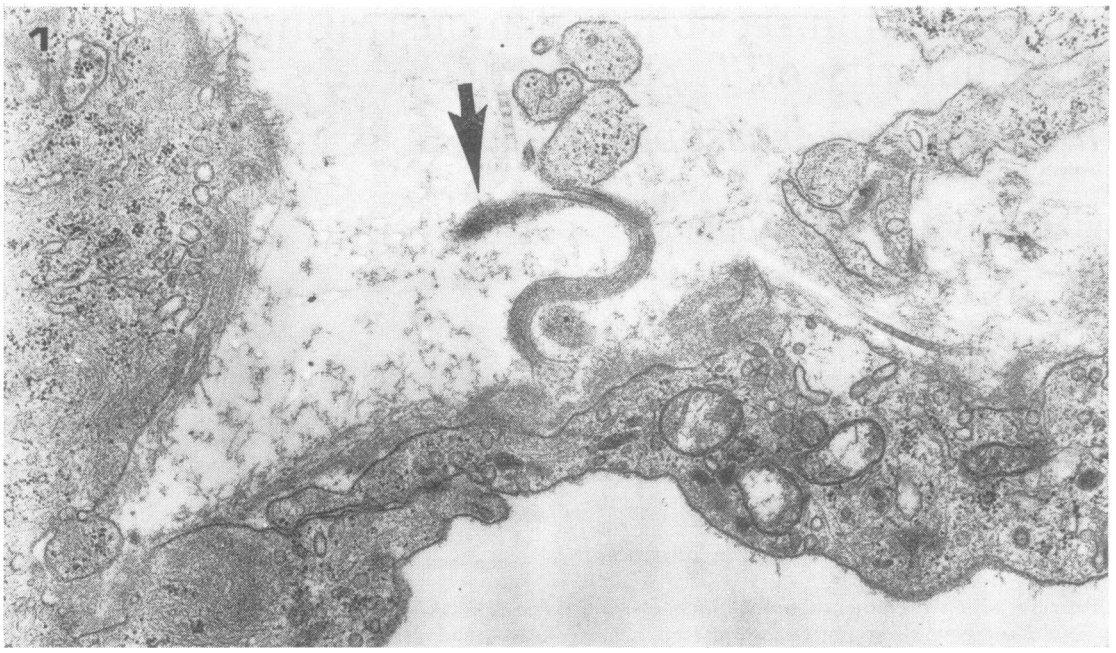
Results

Cells with seemingly intracellular treponemes were extremely rare, and the myriads of invading *Treponema pallidum* were almost exclusively extracellular. They were concentrated around fibroblasts and mesenchymal cells (Fig. 1), and were observed around the interstitial cells of Leydig and small blood vessels, but were never found within seminiferous tubules. The pathogens were also seen within the lumina of capillaries (Fig. 2) and small venules.

The inflammatory response was moderate and noteworthy because of the disproportionately high number of plasma cells in the testicular interstitium.

Although they were few in number in relation to the large number of extracellular organisms, it was not difficult to find treponemes included in cytoplasmic folds and invaginations. Some of these appeared to be intracellular until serial sectioning proved them to be otherwise (Figs 3 to 6).

Figs 7 to 11 are micrographs of serial sections of a of a fibroblast within which a treponeme appears. Figs 7 and 11 are from sections taken beyond each



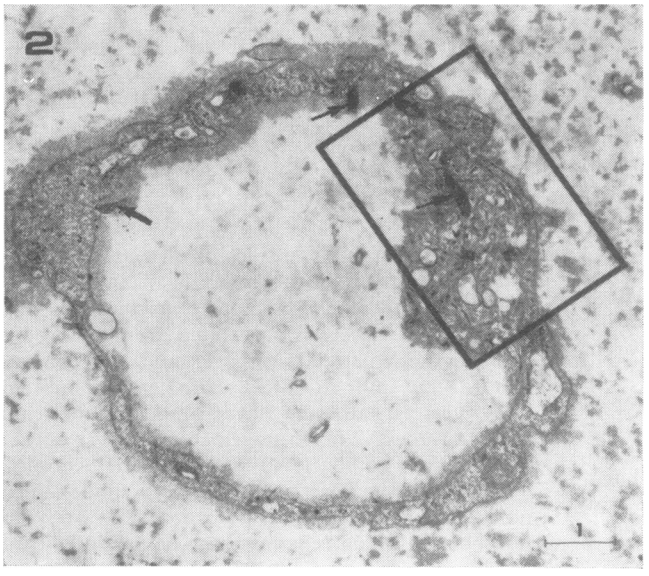


FIG. 1 *A treponeme (arrow) is seen coiling in the extracellular space close to a fibroblast (FB). Fibrils and ribosomes can be seen in the spirochaete, but the typical spirals are absent. Chrome-osmium fixative.* × 25,000

FIG. 2 *Spirochaetes appear inside the lumen of a capillary. The endothelial cell appears to have been invaded by a treponeme but serial sectioning was not done so that the intracellularity could be clearly defined. Gluteraldehyde-cacodylate fixative.* × 10,000

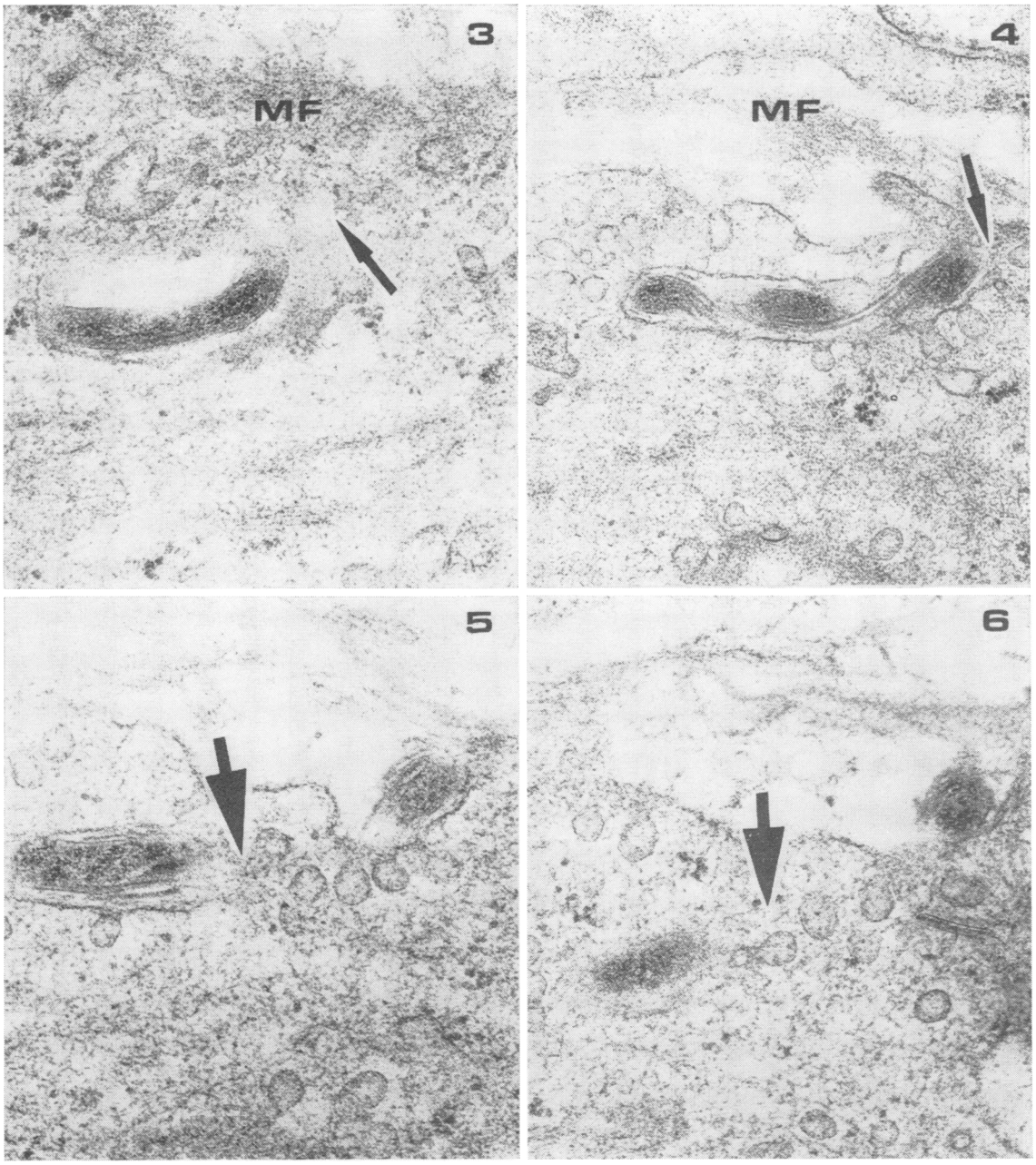
end of the organism; these indicate that the spirochaete is entirely enclosed by cellular matrix and may be considered to be intracellular.

The intracellularity of *T. pallidum* was also demonstrated by serial sectioning in a Leydig cell (Fig. 12), in a monocyte, and within mesenchymal cells (Fig. 13).

Multiple bacterial invasion of a single-cell type was seen several times. In Fig. 13, two out of four treponemes found in a single mesenchymal cell are visible.

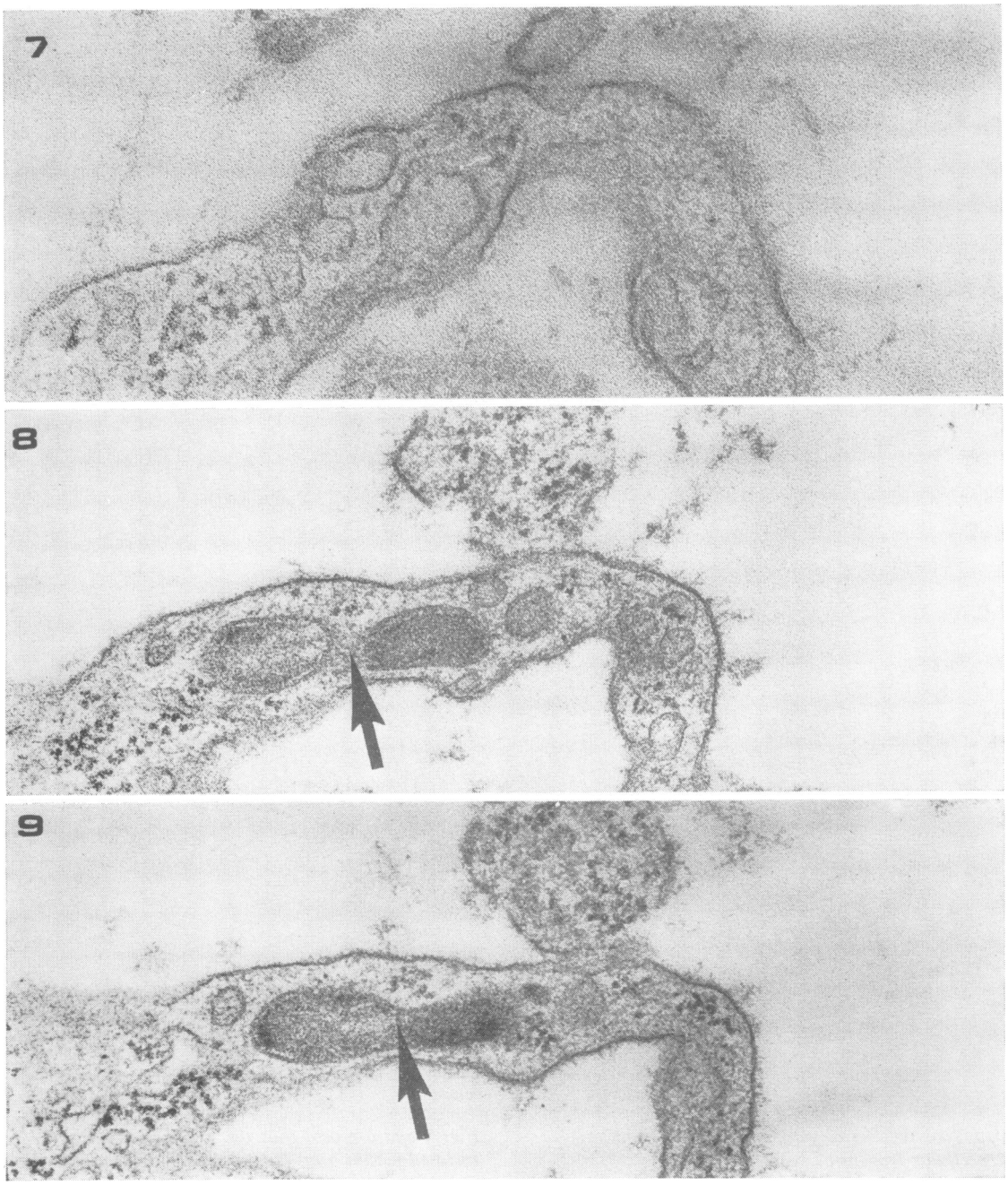
Serial sections could not be obtained for examination of the capillary endothelial cell of Fig. 2, but a treponeme appears to be located within the cell.

The morphology of the extracellular spirochaetes generally conformed to previous descriptions



FIGS 3 to 6 *Serial sections of an apparent intracellular treponeme.*
In Figs 3 *and* 4 *the spirochaete appears to be located within a vacuole in the cell cytoplasm. A diffuse edge of microfibrils (MF) (Fig.* 3*) is sectioned through to reveal a typical membranous edge of the fibroblast (Fig.* 4*)* × 67,000

Figs 5 *and* 6 *reveal that the treponeme had been enclosed within a membranous invagination and in these figures the single spirochaete appears as two; one intracellular and one extracellular. The arrow indicates the position of the overlapping cellular edge. The presence of axial filaments in Figs* 3 *and* 4 *are more characteristic of extracellular treponemes.* × 100,000



FIGS 7 *to* 11 *This series reveals a spirochaete (arrow) enclosed within a fibroblast. The cytoplasm remains uninterrupted by an entering treponemal process at the beginning (Fig.* 7*) and end (Fig.* 11*) of the series. The host cell cytoplasmic membrane is intact throughout the series, thus fulfilling the definition of intracellularity. Chrome-osmium fixative.* × 62,500

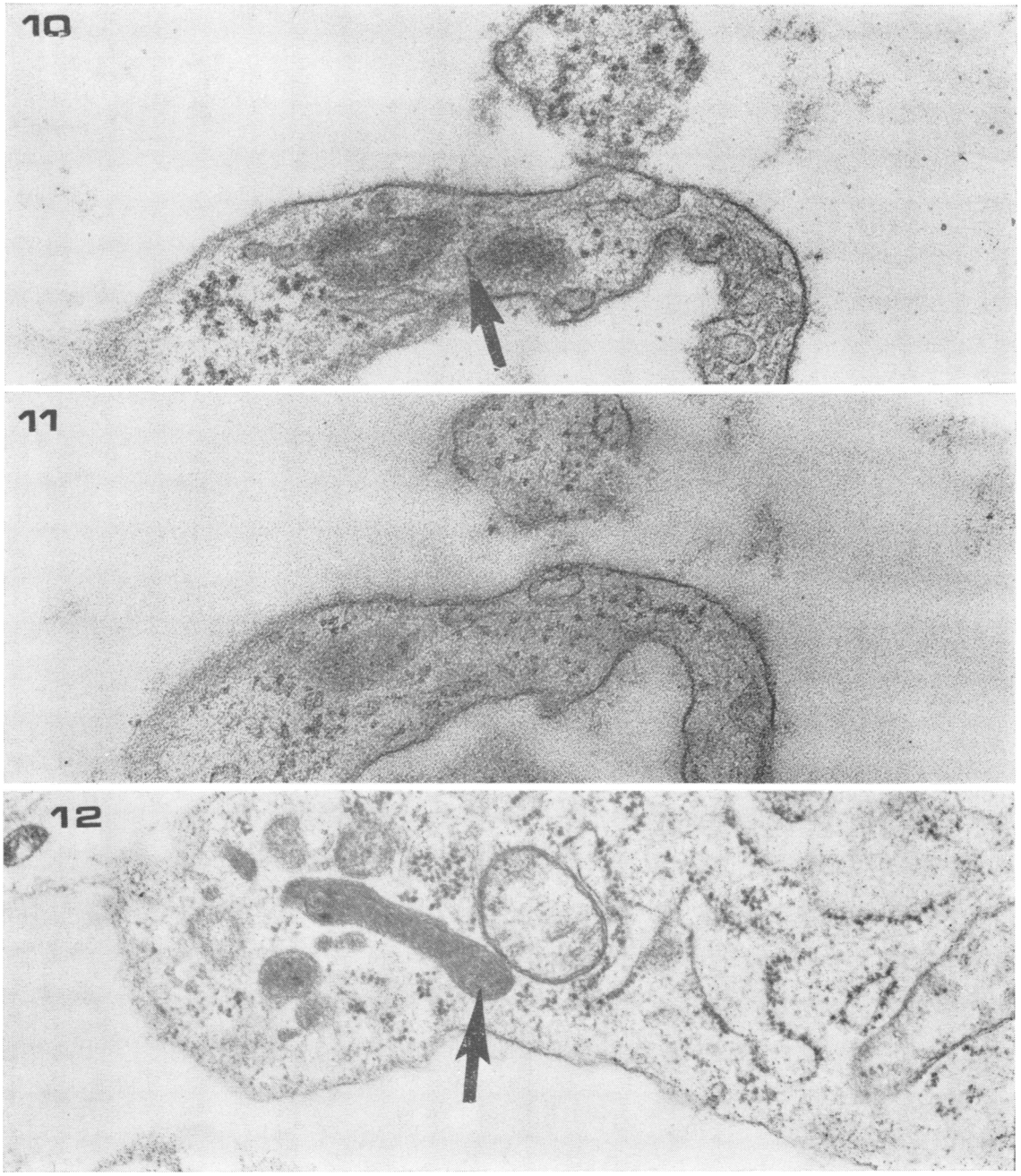


FIG. 12 *Leydig cell containing a spirochaete (arrow). This cell was serially sectioned and the treponeme was found to be intracellular. Chrome-osmium fixative.* × 37,500

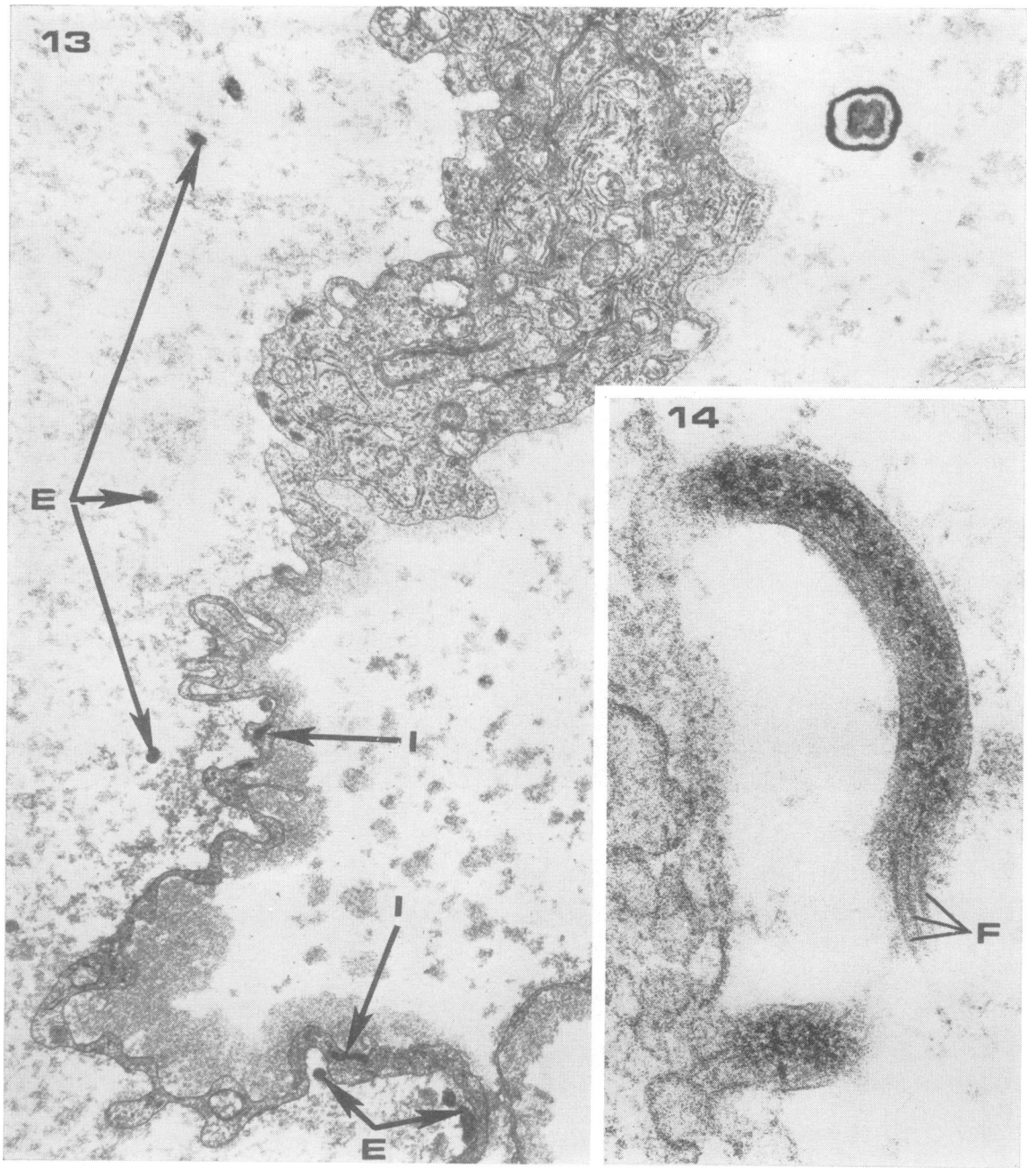
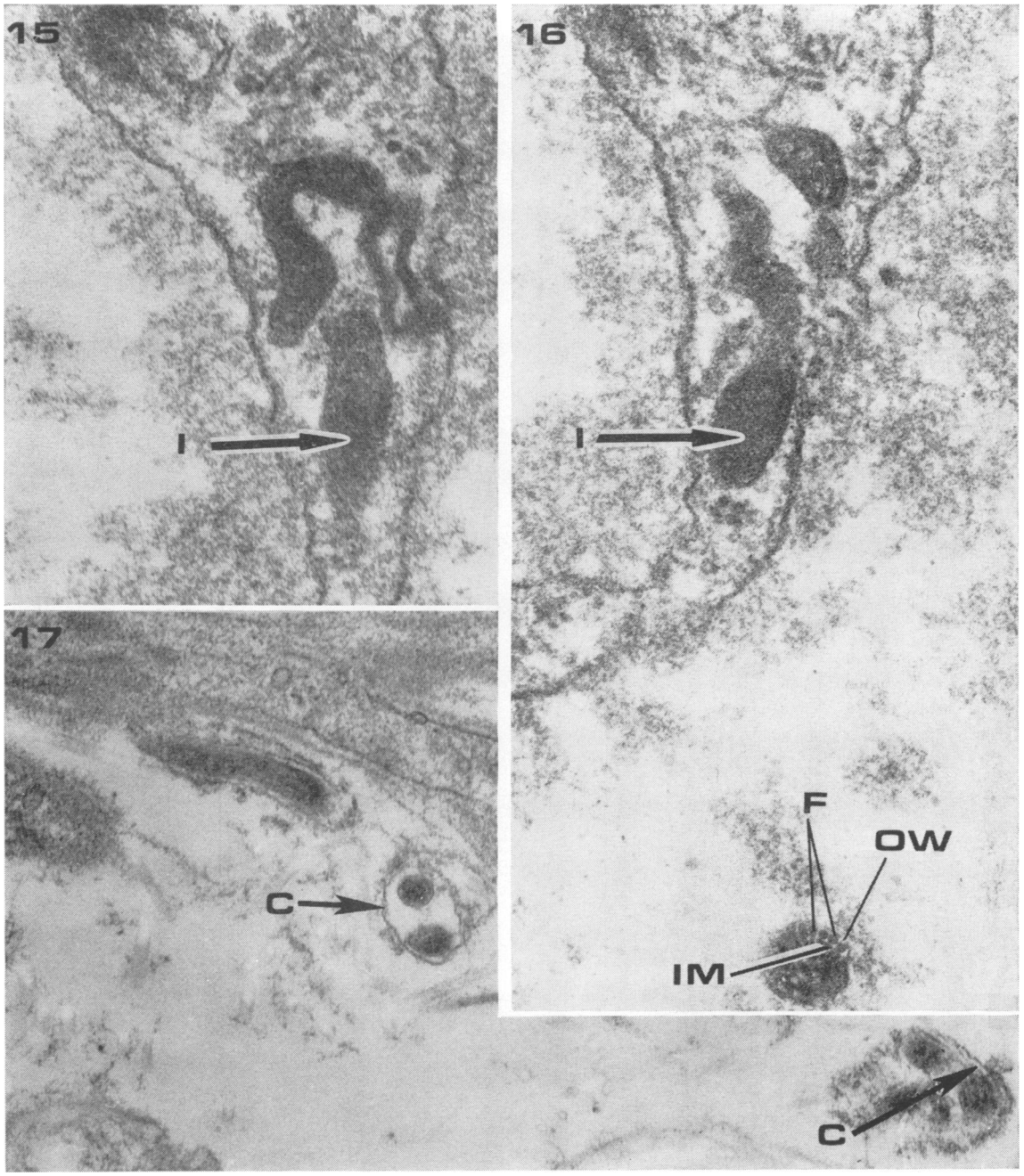


FIG. 13 *This mesenchymal cell was found upon serial observation to contain four treponemes. Two of the organisms are visible in the plane of sectioning. (E, extracelleluar treponemes; I, intracellular treponemes.) Gluteraldehyde-cacodylate fixative.* × 12,500

FIG. 14 *An extracellular treponeme appears to be burrowing into a mesenchymal cell. Typical spirals are not present, but ribosomes and fibrils (F) are clearly seen. Chrome-osmium fixative.* × 100,000



FIGS 15 *and* 16. *Two examples from a series demonstrate the shortened, thickened, electron dense, folded appearance of the intracellular treponemes (I). An extracellular organism exhibiting typical fibrils (F), and an inner membrane (IM) and outer cell wall (OW), appears in the same micrograph (Fig.* 16*). Gluteraldehyde-cacodylate fixative.* × 75,000

FIG. 17 *Encystment (C) appears to have begun in the extracellular treponemes. Chrome-osmium fixative.* × 37,500

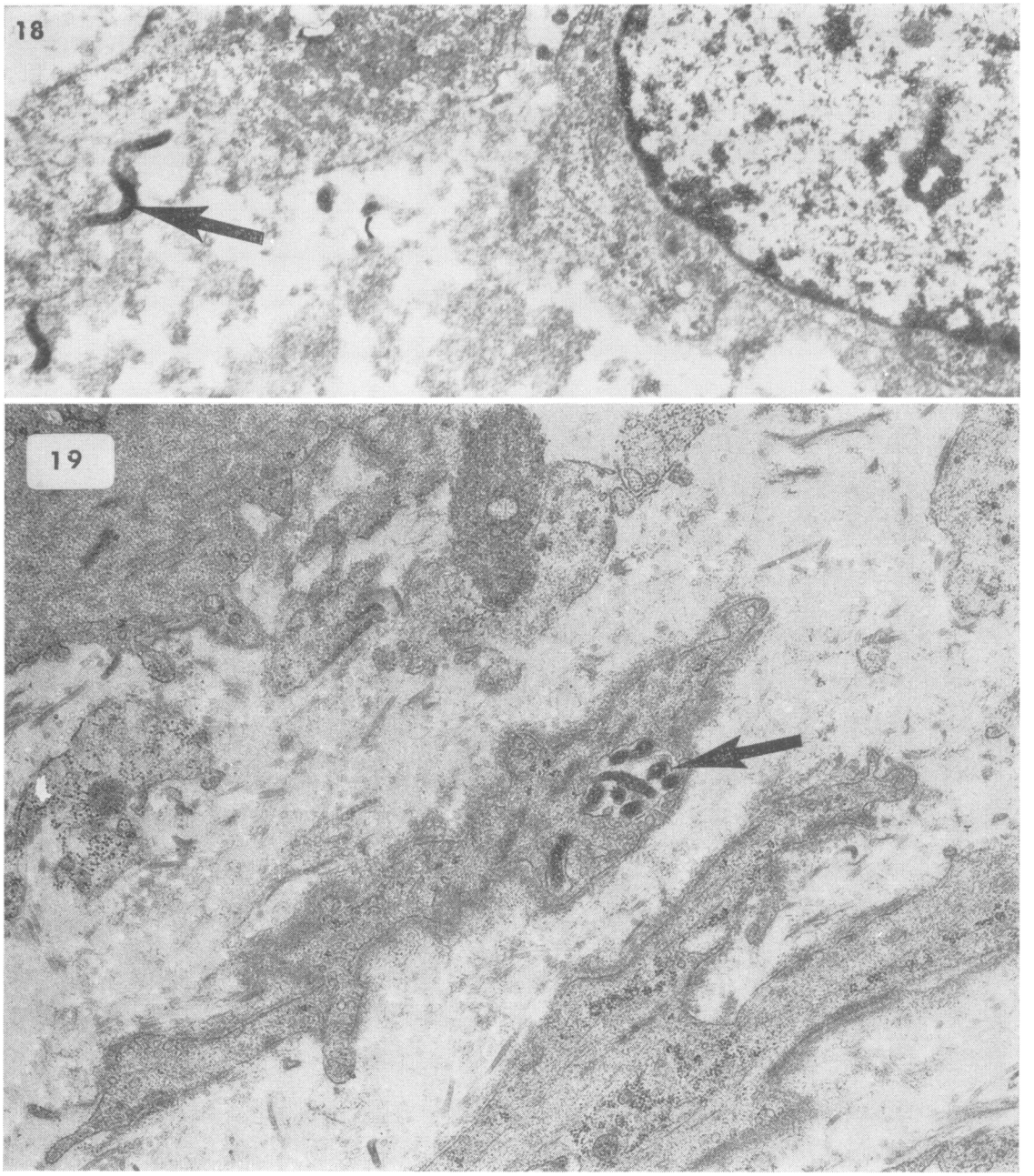


FIG. 18 *Extracellular treponemes were found which exhibited typical spiral coils such as that (arrow) appearing close to the processes of a mesenchymal cell. Gluteraldehyde-cacodylate fixative.* × 12,500

FIG. 19 *Intracellular encystment (arrow) appears to have surrounded one highly convoluted or several spirochaetes. This cell was not serially sectioned in order to validate the intracellularity. Chrome-osmium fixative.* × 22,500

regardless of the fixation technique utilized. They ranged in size from 0·10 to 0·15μ in diameter and 5μ in length. The characteristic spirals were rarely seen and were especially sparse in organisms which were close to host cells (Figs 1 and 14). A few extracellular treponemes were observed in the interstitium with regular spirals (Fig. 18). These spirals possessed a wavelength of 1·1μ and amplitude of 0·3–0·4μ.

Intracellular treponemes differed from extracellular ones in that they were shorter and thicker (1–1·7μ long and 0·35–0·5μ in diameter). They also lacked the spiral coils and were frequently folded upon themselves (Figs 7, 11, 15, and 16). These intracellular *T. pallidum* organisms were generally more electron dense with less distinct ribosomes and nuclear areas. Inner and outer fibril bundles were never seen associated with the treponemes within cells. In contrast, extracellular treponemes in the same sections showed characteristic morphology (Figs 1, 3–6, 14, 16, 17).

Apparent treponeme encystment was seen, but serial sections were not available to demonstrate this phenomenon (Figs 17 and 19).

Two fixatives were used and compared. Dalton's chrome-osmium fixative appeared to preserve treponemal structure well and allowed definition of ribosomes, fine filamentous nuclear areas, and double cell membranes. But some electron dense cytoplasmic components of the host cells appeared to be lost with this type of fixative. The gluteraldehyde-cacodylate buffer fixative did not appear to preserve a fine, sharp, double unit membrane in all instances, giving the treponeme and host cell a general electron dense appearance. Cytoplasmic details of the host cell, such as fine fibrils and filaments, which were apparent with this fixative appeared to have been lost in the chrome-osmium fixed tissues.

Discussion

The serial section approach has confirmed that *T. pallidum* does become intracellular. The morphology of the organisms in the intracellular location differs from that of those outside the cell. The intracellular organisms are smaller and more electron dense and lack fibrils. These changes were found in several different infected rabbits and with the use of two different fixation techniques. The extracellular treponemes in these experiments, which exhibited morphology in general agreement with the descriptions of other investigators (Ovcinnikov and Delektorskij, 1968, 1969a; Jepsen, Hougen, and Birch-Andersen, 1968) were found on the same sections as the atypical intracellular organisms described herein.

It may be that the intracellular organisms do not have pathogenic potential and that this condition is terminal and short-lived. But treponemes in different stages of lysis were seen only in monocytes and polymorphonuclear leucocytes—cells known for their phagocytic function. The treponemes within fibroblasts always had uniform morphology.

This report of intracellular treponemes should stimulate consideration of the possibility that *T. pallidum* may be 'stored' intracellularly, with retention of its antigenicity, viability, or even its pathogenicity, in some host cells. These suggestions have been made by Abe (1967) and by Ovcinnikov and Delektorskij (1969b) after they reported finding *T. pallidum* in plasma cells. Other workers (Azar and others, 1970) have also reported the finding of treponemes in plasma cells; although plasma cells were abundant in the inflammatory process of our experiment, we saw no organisms within them. The speculation of Goldman (1969, 1971) that an intracellular habitat may provide another protective device for the treponemal invader against the action of drugs or the immunological reactions of the host is raised once more.

Cyst-like forms, as described by Ovcinnikov and Delektorskij (1968, 1969a), were seen in our preparations. No serial sectioning was performed on the specimens in Figs 17 and 19 in order to assess the possibility of intracellular encystment, the number of spirochaetes within a single cyst, and whether the cyst wall fully enveloped the treponemes. The appearance of *T. pallidum* in small vessels has been previously described in older lesions but not early in the development of the infection as in these experiments, and this may indicate why the early treatment of a syphilitic infection is of clinical importance.

Summary

Syphilitic rabbit orchitis was studied electron microscopically, using serial sectioning of cells which appeared to contain treponemes in order to prove true intracellularity. The organism was shown to invade a variety of types of host cell and it was observed that morphological changes occurred in the spirochaetes upon cell invasion.

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Coupes ultra-fines en série démontrant la présence intra-cellulaire de *T. pallidum:* Étude au microscope électronique

SOMMAIRE

L'orchite syphilitique du lapin fut étudiée au microscope électronique par la technique de coupes en séries de cellules paraissant contenir de tréponèmes afin de prouver leur présence intra-cellulaire. L'organisme se montra envahir une variété de types de cellules de l'hôte et il fut observé que des changements morphologiques survenaient chez les spirochètes lors de l'invasion cellulaire.